Two New Species of *Zygonemertes* (Nemertea: Enopla: Monostilifera) from Hokkaido, Japan

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Two new species of the marine monostiliferous hoplonemertean genus *Zygonemertes* (Nemertea: Enopla) are fully described and illustrated from specimens collected in Hokkaido, northern Japan. *Zygonemertes shintai* sp. nov. is characterised by a posteriorly lobed central stylet basis, ten proboscis nerves, and anterior pouches of the intestinal caecum that do not reach the ganglionic lobes of the brain. *Zygonemertes jamsteci* sp. nov. is distinguished from the most similar species, *Z. virescens* (Verrill, 1879), by having a proboscis as long as the body and a mixed type of pre-cerebral septum. All stages of oogenesis are present simultaneously in mature females of *Z. jamsteci*. Their oocytes seem to originate from the ganglionic cells of the lateral nerves. All six sectioned specimens of *Z. jamsteci* were infected by large numbers of unidentified acephaline eugregarines.
Key Words: Nemertea, Enopla, Hoplonemertea, *Zygonemertes*, new species, acephaline Eugregarina.

Introduction

Montgomery (1897a) redescribed *Amphiporus virescens* Verrill, 1879 and transferred it to a new genus, *Zygonemertes*, mainly on the basis of its post-cerebral distribution of ocelli. *Zygonemertes* now comprises 17 species distributed almost world-wide; in Japan, however, only one species, *Z. glandulosa* Yamaoka, 1940 has hitherto been known (Yamaoka 1940).

Two species of *Zygonemertes* were found during recent faunal surveys around Hokkaido, Japan. Subsequent histological studies revealed that they represent previously unknown taxa.

Materials and Methods

Specimens for histological examination were anaesthetized in 7.5% $MgCl_2$, fixed in Bouin's solution for 24 hours, embedded in 56–57°C m.p. paraffin wax, and

sectioned at 6 μm before being stained with Mallory trichrome method. Observations on the ocelli, epidermal sickle-shaped bodies, intestinal caecal appendages, and stylet apparatus were made by the squeezing method described by Kirsteuer (1967). The type material is deposited in the Zoological Institute, Hokkaido University, Japan (ZIHU).

Systematics
Family **Amphiporidae** McIntosh, 1874
Genus *Zygonemertes* Montgomery, 1897
Zygonemertes shintai sp. nov.
(Figs 1–5)

Etymology. The new species is named in honour of Mr. Kazuro Shinta, the administrant of Oshoro Marine Biological Station, Hokkaido University since 1944, as a tribute to his constant, devoted support for so many researchers in their field activities.

Type locality. Oshoro, Hokkaido, Japan; intertidal, among the blue mussels, *Mytilus galloprovincialis* Lamarck, 1819.

Material examined. All the specimens were collected from the type locality: two specimens, 30 July 1996; five specimens, 2 July 1998; four specimens, 6 September 2000; two specimens, 3 March 2001. Holotype: 2 July 1998, immature, full series of transverse sections, 16 slides, ZIHU-1926. Paratypes: 2 July 1998, immature, full series of transverse sections, 31 slides, ZIHU-1927; 3 March 2001, two whole specimens, preserved in 100% EtOH, ZIHU-2105 and ZIHU-2106.

Description. *External features*: The body is slender, filiform, and possesses an almost uniform width throughout its length (Fig. 1A). In life the specimens varied in length from 4 to 20 mm and in width from 0.4 to 0.6 mm. The dorsal surface of the body is green, while the ventral side is variously dark yellow to dark orange. In life the cerebral ganglia were distinguishable by their orange hue throughout the integument. The head is anteriorly rounded and wider than the adjacent body. There are two pairs of cephalic furrows on the head; the anterior furrows curve forward on the ventrolateral surfaces, while the posterior furrows completely encircle the head, forming a posteriorly directed V-shape on its dorsal surface. The eyes are numerous and more or less arranged in four rows (Fig. 1B). Behind the posterior cephalic furrows there is also a row of three to eight smaller eyes on each side of the body. The posterior end of the body is slightly pointed.

Body wall, musculature, and parenchyma: The ciliated epidermis (Figs. 2A, 3) is mostly 20–30 μm thick in the vicinity of the brain. It contains numerous minute, sickle-shaped bodies (Fig. 2B) like those reported from several species of *Zygonemertes* but also found in *Quequenia gracilis* Moretto, 1974, *Alaonemertes michaelseni* Stiasny-Wijnhoff, 1942, and *Emplectonema echinoderma* (Marion, 1873) (Moretto 1974; Stiasny-Wijnhoff 1942; Montgomery 1897a).

The dermis (Fig. 3) is thin but distinct, attaining a thickness of 2–3 μm in the vicinity of the brain. Its outer surface is developed into cup-like concavities (Fig. 3), as described for hoplonemertean species by Norenburg (1985).

The body-wall musculature (Fig. 3) is moderately well developed. In the region of the foregut the outer circular layer is 8–12 μm thick, and the inner longitudinal
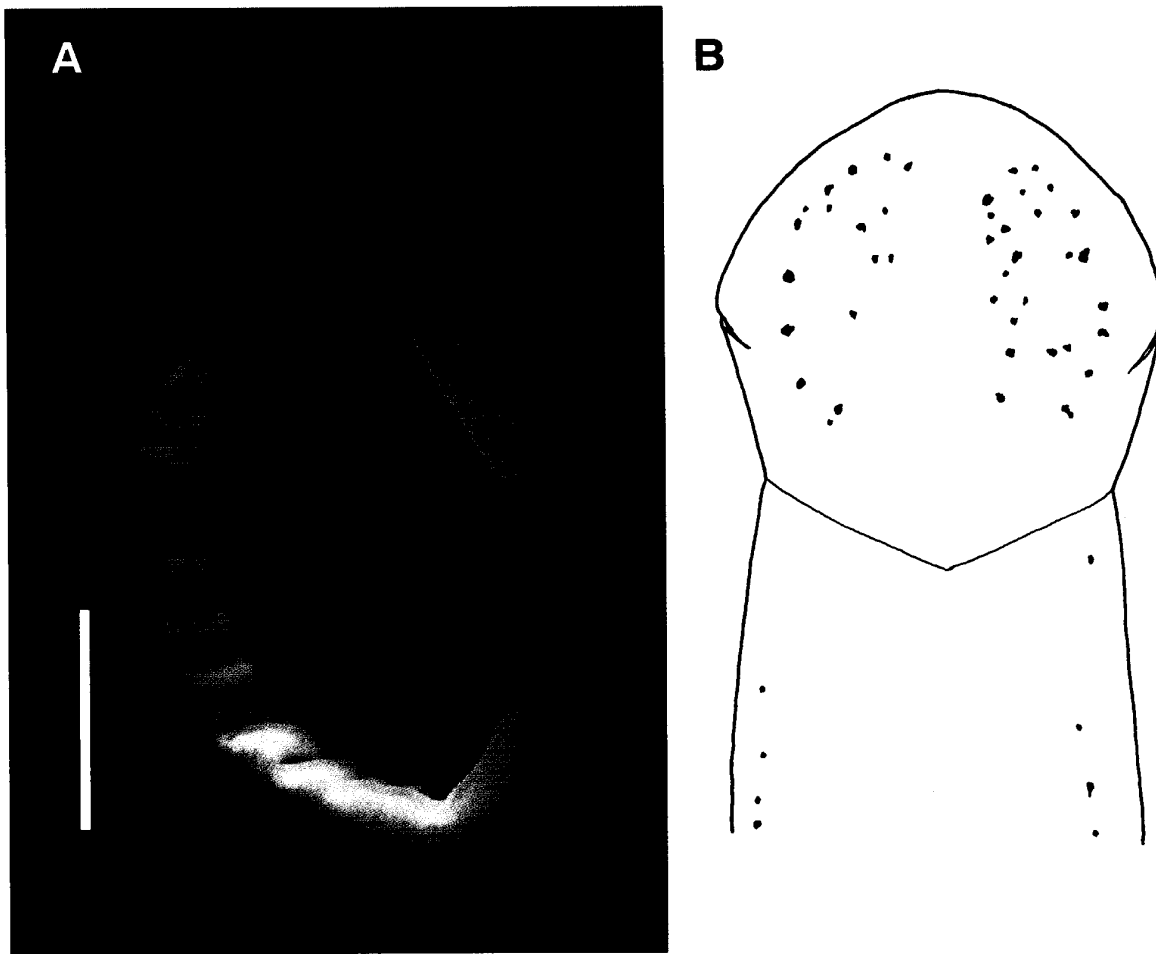
Fig. 1. *Zygonemertes shintai* sp. nov. A, photograph of a living specimen; B, enlargement of the head to show the arrangement of the eyes and the cephalic furrows. Scale bar (refers to A only)=3 mm.

layer 15–25 μm thick. The longitudinal layer is anteriorly divided only in the cerebral region. In the region surrounding the middle of the brain the inner portion of the longitudinal muscle layer comprises thin but discrete bundles of fibres, which are separated from the outer zone by a thin layer of parenchymatous connective tissue. Further forward these bundles show a tendency to fuse with each other but never form a continuous, complete layer. In the anterior vicinity of the brain the inner portion of the longitudinal muscles in the dorsal and ventral halves of the body are separated from the outer layer by the posterior extension of the cephalic glands (Fig. 2C), with the inner and outer layers attaining 5–15 μm and 20–30 μm thick, respectively. In front of the brain all of the fibres from the inner portion turn inward to form a proboscis insertion, but the dorsal and lateral portions of the outer layer also send fibres to the insertion (Fig. 2D); according to Gibson's (1990) definition, the pre-cerebral septum in this species is of the mixed type. Pre-cerebrally, some fibres from the outer portion of the longitudinal muscle layer turn inward to form cephalic retractors.

The diagonal muscle layer is weakly developed between the outer circular and

Table 1.   Body lengths, central stylet and basis lengths, and ratios of central stylet length to basis length recorded for three specimens of *Zygonemertes shintai* sp. nov. collected in 6 September 2000. These specimens were consumed after *in vivo* observations; the data for the type specimens were thus not included below.

Specimen No.	Body length (mm)	Central stylet length (μm)	Basis length (μm)	Stylet to basis ratio
1	16	110	180	0.61
2	20	120	250	0.48
3	12	90	170	0.53
Mean	16	107	200	0.54

inner longitudinal muscle layers in the anterior region of the body (Fig. 2A). Dorsoventral muscle bundles are not found in any body region.

Parenchymatous connective tissue is sparingly formed throughout the body.

Proboscis apparatus: The proboscis pore is sub-terminal. It leads into a slender, tubular rhynchodaeum. The rhynchodaeal epithelium, 2–5 μm thick, possesses neither cilia nor gland cells. After the oesophagus branches from its ventral floor, the rhynchodaeum begins to be surrounded by a delicate layer of circular muscle fibres that are about 8 μm thick, but which suddenly expand in diameter just in front of the proboscis insertion.

The rhynchocoel extends almost to the hind end of the body. Its wall contains separate outer circular and inner longitudinal muscle layers.

The proboscis is rather short and slender. Its anterior portion, the diameter of which reaches up to about 35–45% of the body diameter, contains three muscle layers (outer and inner circular, middle longitudinal); ten distinct proboscis nerves extend through the longitudinal muscle layer (Figs 2E, 3).

The stylet bulb region has a normal monostiliferous construction. The stylet basis is cylindrical and posteriorly lobed (Fig. 2F). The stylet apparatus was observed in three specimens in life (Table 1). The central stylet is smooth and 90–120 μm long, its basis 170–250 μm long. The stylet/basis ratio varies from 0.48 to 0.61 with an average value of 0.54. There are two accessory stylet pouches, each containing two to four accessory stylets.

Fig. 2. *Zygonemertes shintai* sp. nov. A, transverse section to show the appearance of the body wall (arrows indicate the diagonal muscle layer); B, photomicrograph of the epidermis of a squeezed specimen, taken in life (an arrow indicates a sickle-shaped body); C, transverse section through the brain to show the divided nature of the body-wall longitudinal musculature (arrows indicate the inner portion of the divided longitudinal muscle layer); D, transverse section through the proboscis insertion (arrows indicate muscle fibres from the outer portion of the longitudinal muscle layer); E, transverse section through the anterior proboscis (arrows indicate the 10 proboscis nerves); F, photomicrograph of a typical stylet and basis, taken in a living specimen. Abbreviations: cg=cephalic gland; cm=circular muscle layer; dc=dorsal cerebral commissure; de=dermis; ep=epidermis; lm=longitudinal muscle layer; oe=oesophagus; vc=ventral cerebral commissure. Scale bars: A=10 μm; B, D=25 μm; C, E, F=50 μm.
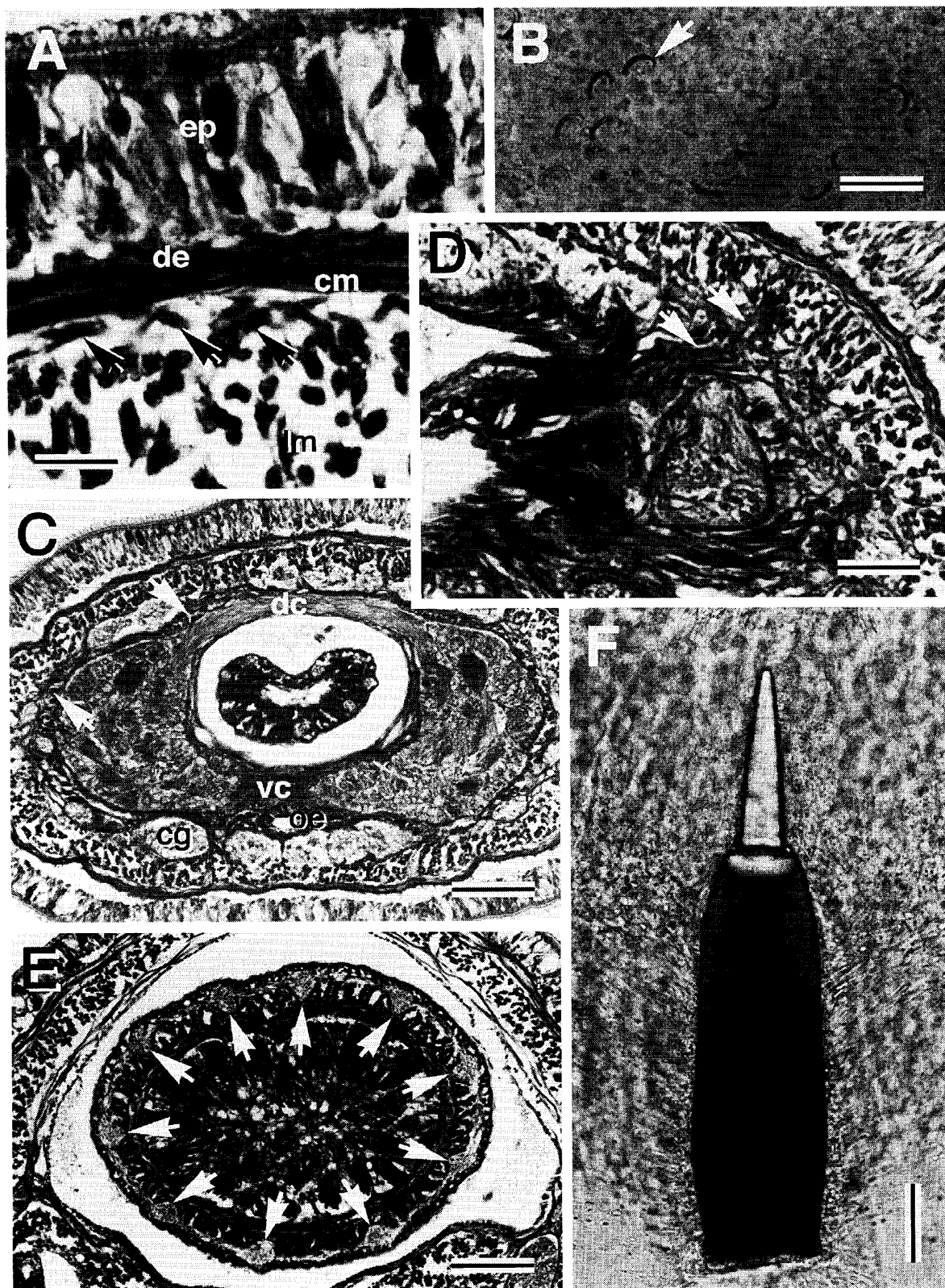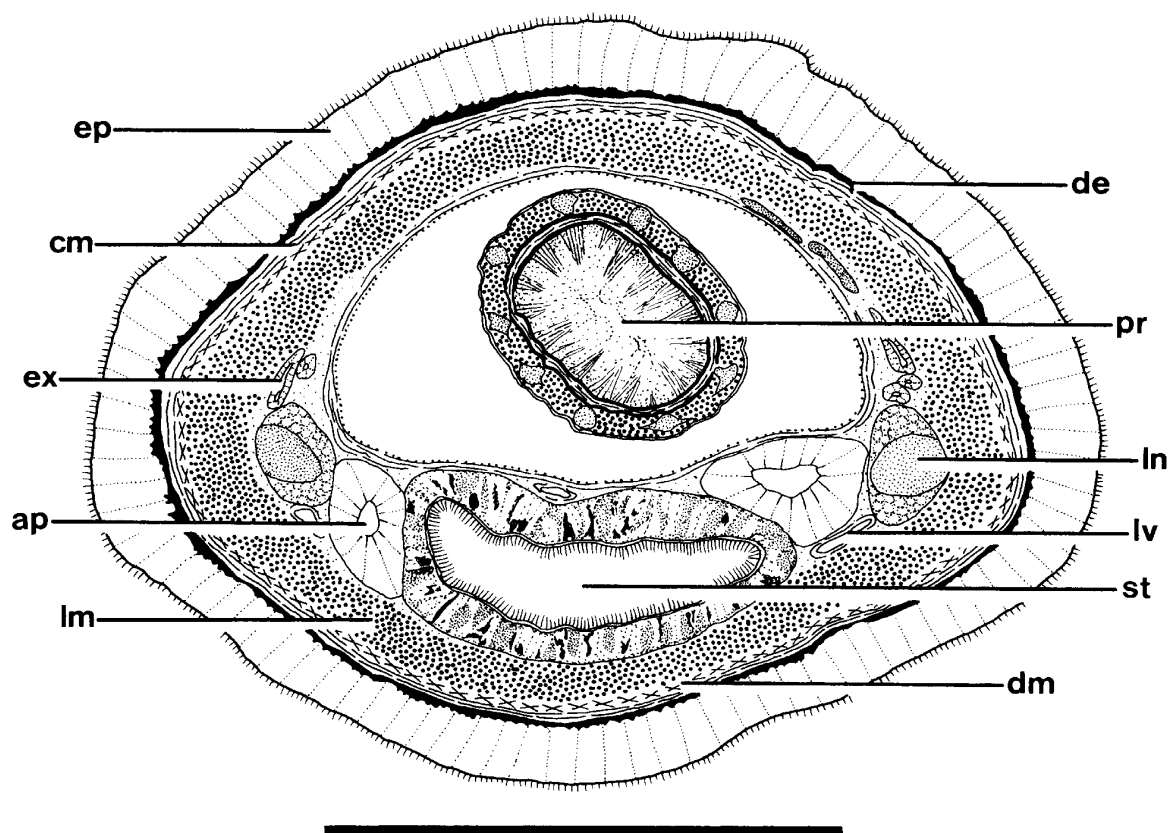
Fig. 3. *Zygonemertes shintai* sp. nov. Transverse section through the foregut region to show the arrangement of the various body components. Abbreviations: ap = anterior pouch of the intestinal caecum; cm = circular muscle layer; de=dermis; dm=diagonal muscle layer; ep=epidermis; ex=excretory collecting tubule; lm=longitudinal muscle layer; ln=lateral nerve cord; lv=lateral blood vessel; pr=proboscis; st=stomach. Scale bar=300 μm.

The posterior part of the proboscis possesses no unusual features.

Alimentary canal: The oesophagus emerges from the rhynchodaeum just in front of the proboscis insertion. It is short and proximally invested with longitudinal muscle fibres, and its epithelium is 5–8 μm thick, possessing neither cilia nor gland cells. The onset of the stomach is marked by the appearance of cilia and basophilic gland cells. Two portions of the stomach (Fig. 3) are histologically distinguishable by the type of basophilic gland. In the anterior portion the basophilic glands are coarsely granular, while in the posterior portion they contain homogeneous contents. The stomach wall is slightly folded and is mostly up to 25 μm thick. Longitudinal somatic muscle fibres are associated with the stomach wall. Toward its rear the stomach begins to lose its basophilic glands and exhibits a reduction in epithelial height. At the same time it begins to narrow and leads imperceptibly into the pyloric portion of the foregut (Fig. 4A).

From the junction between the pylorus and the intestine a short ventral caecum extends forward (200 μm in the holotype, 60 μm in the sectioned paratype [ZIHU-1927]) to reach under the middle portion of the pylorus (Fig. 4A). The caecum anteriorly bifurcates to form a pair of anterior pouches (Fig. 4B) that run laterally to the pylorus and the stomach but do not reach the brain (Fig. 4C). Both the
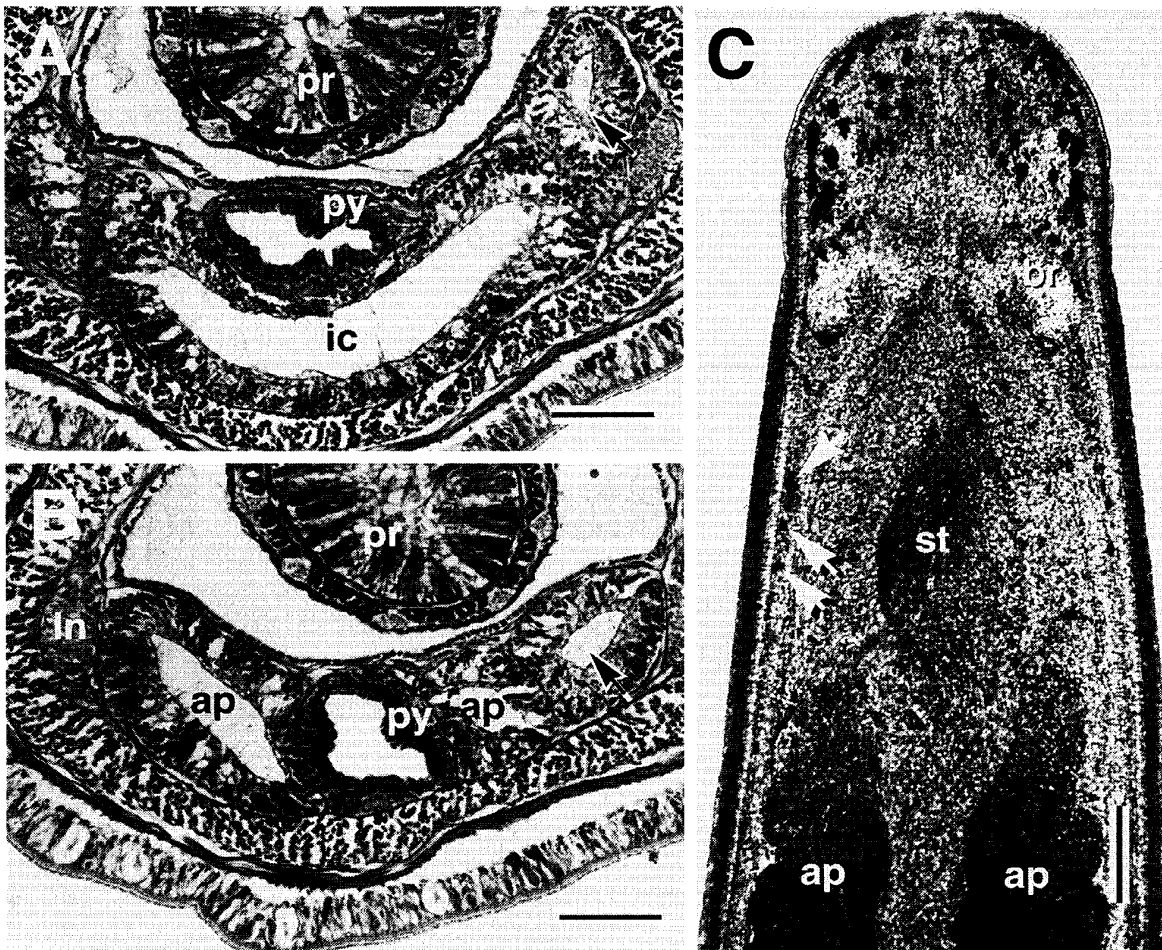
Fig. 4. *Zygonemertes shintai* sp. nov. A, transverse section through the intestinal caecum (an arrow indicates the lateral diverticulum of the intestinal caecum [ic]); B, transverse section through the pyloric region (an arrow indicates the lateral diverticulum of the anterior pouch [ap] of the intestinal caecum); C, photomicrograph of the anterior part of a squeezed specimen to show the anterior pouches [ap] of the intestinal caecum not reaching to the brain [br] (arrows indicate the post-cerebral ocelli). Abbreviations: ln=lateral nerve cord; pr=proboscis; py=pylorus; st=stomach. Scale bars: A, B=50 μm; C=250 μm.

caecum and its anterior pouches possess a few shallow lateral diverticula (Fig. 4A, B). The main intestinal canal possesses a normal construction and bears throughout its length shallowly lobed lateral diverticula. The anus is at the posterior tip of the body.

Blood vascular system: The blood vascular system consists of three primary longitudinal vessels, which meet anteriorly to form a vascular loop above the rhynchodaeum near the tip of the head. There is no posterior connective of the vascular loop in front of the brain. Just posterior to the cerebral ring, the mid-dorsal vessel joins the right vessel. The mid-dorsal vessel immediately enters the ventral floor of the rhynchocoel, forming a vascular plug (Fig. 5A) 20 μm wide and 8 μm tall, and then emerges from the rhynchocoel to continue posteriorly between the rhynchocoel and gut. Both lateral vessels continue to the posterior end of the body

in a ventrolateral position near the lateral nerve cords (Fig. 3), meeting with the mid-dorsal vessel in a supra-intestinal anastomosis near the hind end of the body. No other cross-connectives are present. No evidence of blood corpuscles was found.

Nervous system: The brain is large and well developed, the dorsal and ventral lobes being about the same size. A thin but distinct outer neurilemma ensheathes the cerebral ganglia, but there is no inner neurilemma between their fibrous and neuroganglionic tissues. The dorsal and ventral commissures are respectively 10 μm and 40 μm thick. Behind the ventral commissure, there is an extra sub-oesophageal ventral commissure (Fig. 5B); a similar structure has been reported from some species of *Ototyphlonemertes* (Envall 1996; Gerner 1969; Mock 1978).

The lateral nerve cords contain one or two myofibrillae in the neuropil, but no accessory nerves. They are connected posteriorly by a supra-intestinal commissure near the hind end of the body.

Apical organ and cephalic glands: The small apical organ (Fig. 5C), a ciliated chamber 25 μm in diameter, opens at the tip of the head just above the proboscis pore.

The cephalic glands consist of typical basophilic lobules. Near the tip of the head they are scattered, but more posteriorly they become fused to form two discrete bundles dorsal and ventral to the rhynchodaeum (Fig. 5D). These bundles extend posteriorly beyond the proboscis insertion to the middle portion of the brain.

Sense organs: The eyes are of a pigment cup form, up to 25 μm in maximum dimension. Behind the brain there is also a single lateral row of 3–8 eyes above the lateral nerve cord on each side of the body (Fig. 4C). These post-cerebral eyes are smaller than the pre-cerebral eyes, only up to 15–20 μm in diameter (Fig. 5E).

Compared with the body diameter the cerebral sense organs are large, about 85 μm tall and 60 μm wide (Fig. 5D). They lie immediately in front of the proboscis insertion and open ventrolaterally from the anterior pair of cephalic furrows. The ciliated canals of the cerebral sense organs turn inward posteriorly to become associated with neuroganglionic tissues. The ciliated canals are about 30 μm in diameter and their posterior extremities are bifurcated for a short distance (24 μm) (Fig. 5D).

Excretory system: The excretory system extends from the rear of the brain to the posterior portion of the foregut, situated above the lateral nerve cords beside the rhynchocoel on each side of the body (Figs 3, 5E, F). It consists of small but thick-walled collecting tubules, 10–18 μm in diameter, opening ventrolaterally to the exterior through a pair of slender efferent ducts via a small nephridiopore on each side (Fig. 5F).

Fig. 5. *Zygonemertes shintai* sp. nov. A, transverse section through the vascular plug (arrowed); B, transverse section to show the sub-oesophageal extraventral commissure (arrowed); C, transverse section through the apical organ [ao]; D, transverse section through the pre-cerebral region to show the cerebral sensory organ [co] (arrows indicate the posteriorly bifurcated cerebral canals); E, transverse section through the stomach region to show a post-cerebral ocellus (arrowed); F, transverse section to show an excretory efferent duct (arrowed). Abbreviations: cg=cephalic gland; ex=excretory collecting tubule; ln=lateral nerve cord; oe=oesophagus; rc=rhynchocoel; rd=rhynchodaeum; vg=ventral ganglion. Scale bars: A, B, E, F=25 μm; C, D=50 μm.
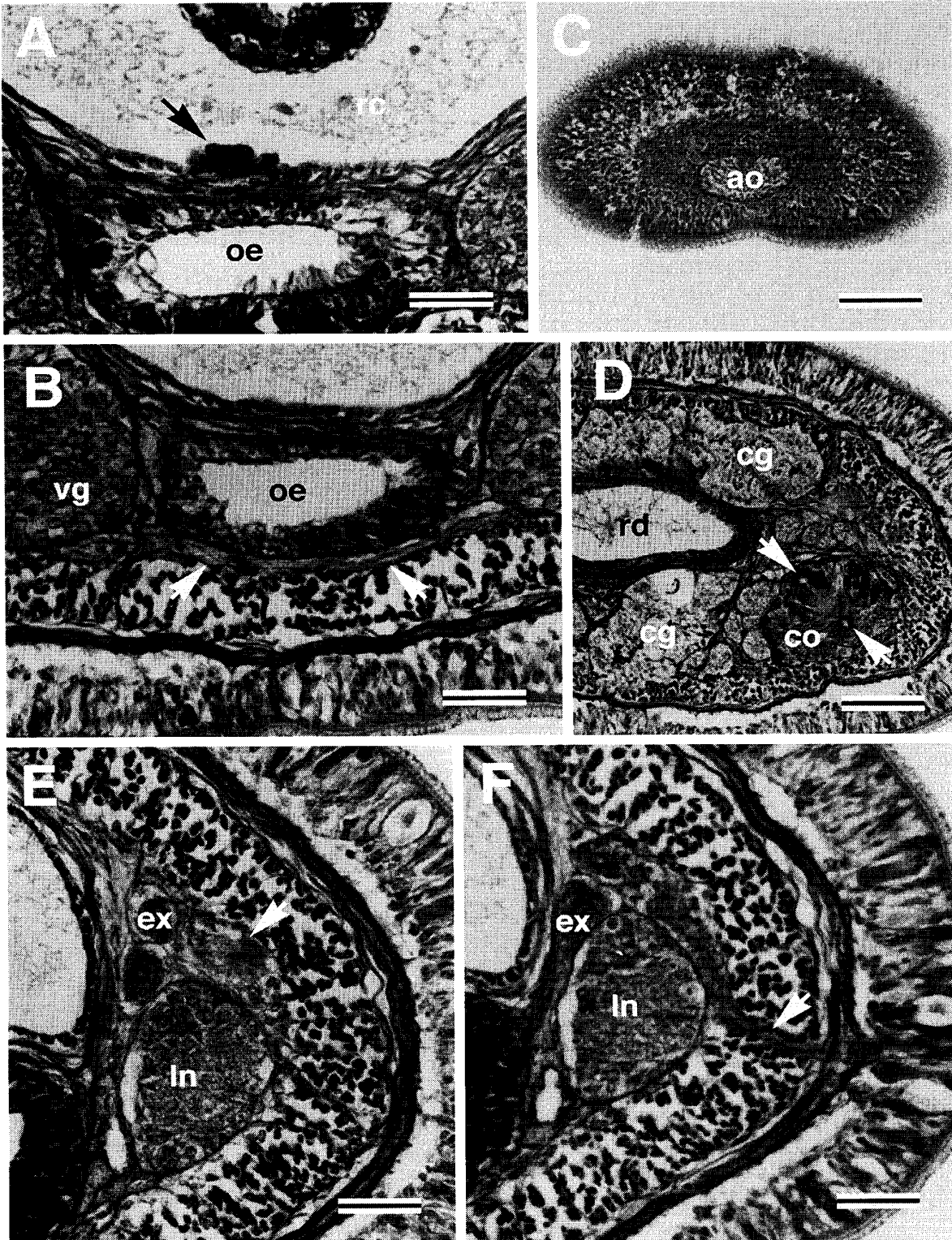
Reproductive system: Both of the sectioned specimens were immature. The gonads are situated dorsally to the lateral nerve cords beside the rhynchocoel on each side. The gonoducts pass above the lateral nerve cords.

Remarks. The most striking feature in this species is the post-cerebral distribution of ocelli. Of the 97 monostiliferous hoplonemertean genera known at present (Gibson 1995; Sundberg and Gibson 1995; Rogers *et al.* 1996; Chernyshev 1998; Crandall and Gibson 1998; Kajihara *et al.* 2001), only two, *Pheroneonemertes* and *Zygonemertes*, share this unusual character. The monotypic genus *Pheroneonemertes* differs from the Oshoro nemerteans in being both hermaphroditic and ovoviviparous, and in possessing extensive cephalic glands that reach far back behind the brain, a network of capillary blood vessels in the cerebral region, and an excretory system extending for most of the body length (Gibson 1990); therefore, the present nemerteans do not belong to this genus. The characteristic features of the present species, including cephalic glands not extending behind the brain, a simple blood vascular system, an excretory system restricted to the foregut region, epidermal sickle-shaped bodies, and a posteriorly truncated stylet basis, enable it to be in-

Table 2.   Some of the morphological features that can be used to distinguish between the 17 described species of *Zygonemertes* and the present two new species from Hokkaido.

Species	I	II	III	IV	V	VI	VII	References
Z. africana Stiasny-Wijnhoff, 1916	+	?	?	+	0	?	0.28	4, 7
Z. albida Coe, 1901	?	?	?	?	0	?	0.67–0.72	2, 3
Z. algensis (Bürger, 1895)	?	0	10–11	0	0	2	0.40–0.72	11
Z. callaina Korotkevich, 1977	+	?	10–12	+	+	2	0.41–0.49	12
Z. capensis Wheeler, 1934	+	0	13	0	0	0	0.22–0.66	5
Z. cocacola Corrêa, 1961	+?	?	10	+	0	?	0.52	9
Z. fragariae Corrêa, 1954	+	+	10	+	0	2	0.50	8
Z. glandulosa Yamaoka, 1940	?	0	12[a]	+	0	?	0.41	6
Z. isabellae Corrêa, 1954	+	+	11	+	0	2	0.67	8
Z. luederitzi Stiasny-Wijnhoff, 1916	+	+	11	0	0	?	0.27	4, 7
Z. maslovskyi (Czerniavsky, 1880)	+	+	10	+[b]	0	2	0.25–0.33	10
Z. simoneae Corrêa, 1961	+	+	12	+	+	1?	0.70	9
Z. tenuirostris Korotkevich, 1977	?	?	?	?	+	2	0.71	12
Z. thalassina Coe, 1901	+	?	12	+	+[c]	2	0.33–0.50	2, 3
Z. virescens (Verrill, 1879)	+	+	10–11	+	+[c]	2	0.40	1, 3, 9
Z. wadjemupensis Gibson, 1999	0	+	10	+	0	1	?	14
Z. zhenylebedevi Chernyshev, 1991	?	?	12	+	+	2	0.30–0.35	13
Z. shintai sp. nov.	+	+	10	0	+[c]	2	0.48–0.61	present study
Z. jamsteci sp. nov.	+	+	10	+	+[c]	2	0.43–0.58	present study

I, epidermal hook present (+) or absent (0); II, apical organ present (+) or absent (0); III, number of proboscis nerves; IV, anterior pouches of intestine reach (+) or do not reach (0) the brain; V, posterior end of stylet basis lobed (+) or smooth (0); VI, number of transverse or oblique cephalic furrows; VII, ratio of central stylet length to basis length.

References: 1, Montgomery (1897a); 2, Coe (1901); 3, Coe (1905a); 4, Stiasny-Wijnhoff (1916); 5, Wheeler (1934); 6, Yamaoka (1940); 7, Stiasny-Wijnhoff (1942); 8, Corrêa (1954); 9, Corrêa (1961); 10, Müller and Scripcaru (1967); 11, Berg (1976); 12, Korotkevich (1977); 13, Chernyshev (1991); 14, Gibson (1999).

[a] Yamaoka (1940) noted that although the main part of the proboscis contained 12 nerves, 13–17 nerves could be seen in the region close to the proboscis insertion.

[b] Müller and Scripcaru (1967: 40) described, "Diese Taschen erreichen den Hinterrand der Ventralganglien nur in unfixiertem Zustand der Tiere, bei der Fixierung verkürzen sie sich bedeutender Weise."

[c] The posterior rim of the stylet basis is often, but not always, lobed (Coe 1905a; present study).

cluded in the genus *Zygonemertes*.

Seventeen species of *Zygonemertes* are known at present (Table 2). Among these, *Z. algensis* (Bürger, 1895), *Z. capensis* Wheeler, 1934, and *Z. luederitzi* Stiasny-Wijnhoff, 1916 are reported to possess anterior pouches of the intestine that do not reach the ganglionic lobes, as in the present species. The present nemerteans from Oshoro, however, can be distinguished from *Z. algensis* and *Z. capensis* in having an apical organ. Also, the present form can be distinguished from *Z. luederitzi* by its 10 proboscis nerves and stylet/basis ratio ranging from 0.48 to 0.61; in *Z. luederitzi*, the number of proboscis nerves is 11 and the stylet/basis ratio is 0.27. The Oshoro nemerteans are thus placed in a new taxon, *Zygonemertes shintai* sp. nov.

Zygonemertes jamsteci sp. nov.
(Figs 6–9)

Etymology. The specific name refers to the Japan Marine Science and Technology Center (JAMSTEC), whose members kindly helped the sampling of the present nemerteans.

Type locality. Akkeshi Bay, Hokkaido, Japan; about 1 m deep, among the zosteraceous eelgrass, *Zostera marina* Linnaeus.

Material examined. A total of 12 living specimens, 8 July 1997. Holotype: mature male, full series of transverse sections, 43 slides, ZIHU-1928. Paratypes: mature female, serial longitudinal sections of anterior portion of body, 9 slides, ZIHU-1929; mature male, full series of transverse sections, 41 slides, ZIHU-2045; mature male, full series of transverse sections, 36 slides, ZIHU-2046; mature female, serial transverse sections of anterior portion of body, 3 slides, ZIHU-2047; mature female, serial transverse sections of anterior portion of body, 14 slides, ZIHU-2048.

Description. *External features*: The size ranges from 6 to 20 mm in length and 0.5 to 1 mm in width. The head is rounded and slightly flattened, demarcated from the rest of the body by the posterior cephalic furrows. The body is widest in the vicinity of the stomach, tapering posteriorly to a slightly pointed end (Fig. 6A). There are two pairs of cephalic furrows on the head (Fig. 6B); the posterior furrows do not meet ventrally (Fig. 6C). The body color varies from off-white to pale green; the color is dorsoventrally uniform. The eyes are numerous, extending backward behind the brain. The pre-cerebral eyes are arranged in four longitudinal rows, each 10–20 in number, while the post-cerebral eyes are situated along the lateral nerve cords, 5–10 in number.

Body wall, musculature and parenchyma: There are numerous sickle-shaped bodies in the epidermis (Fig. 7A). The ciliated glandular epidermis, 25–30 μm thick anteriorly (Figs 7A, 8), is internally bordered by a thin but distinct dermis 2–3 μm thick (Fig. 8).

The body wall muscle layers are moderately well developed. In the region of the foregut, the outer circular and inner longitudinal muscle layers are, respectively, 7–8 μm and 20–30 μm thick, but reduced posteriorly to 3 μm and 13 μm depending upon the degree of contraction. Dorsoventral muscle bundles are present in the foregut and intestinal regions (Figs 7B, 8). Diagonal muscle could be detected only in the longitudinal sections (Fig. 7C). The body-wall longitudinal muscle layer
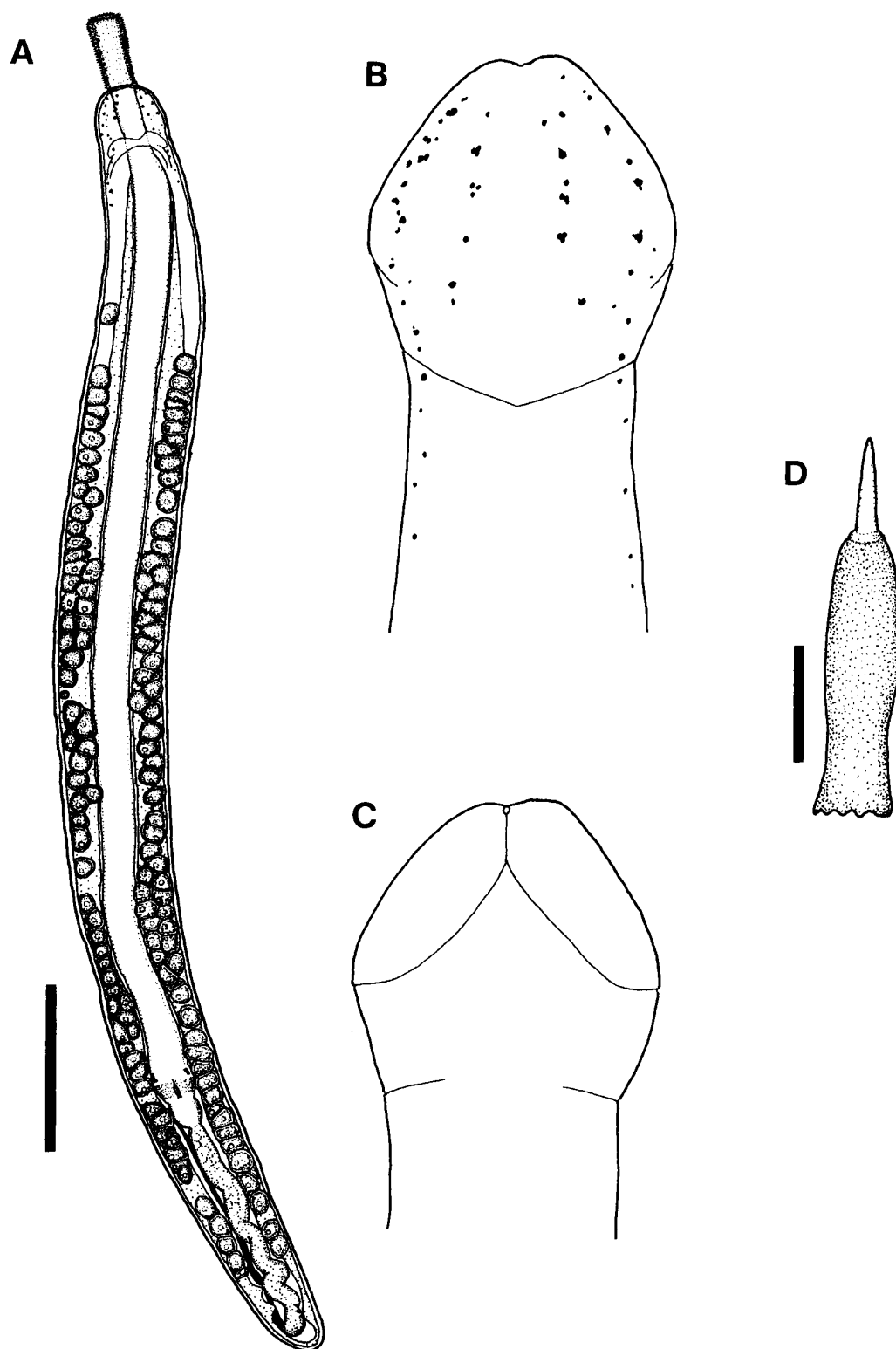
Fig. 6. *Zygonemertes jamsteci* sp. nov. A, drawing of a squeezed specimen, taken in life; B, C, enlargements of the cephalic region in dorsal (B) and ventral (C) views to show the arrangement of the eyes and the cephalic furrows; D, central stylet and posteriorly lobed basis. Scale bars (refers to A and D only): A=4 mm; D=100 μm.
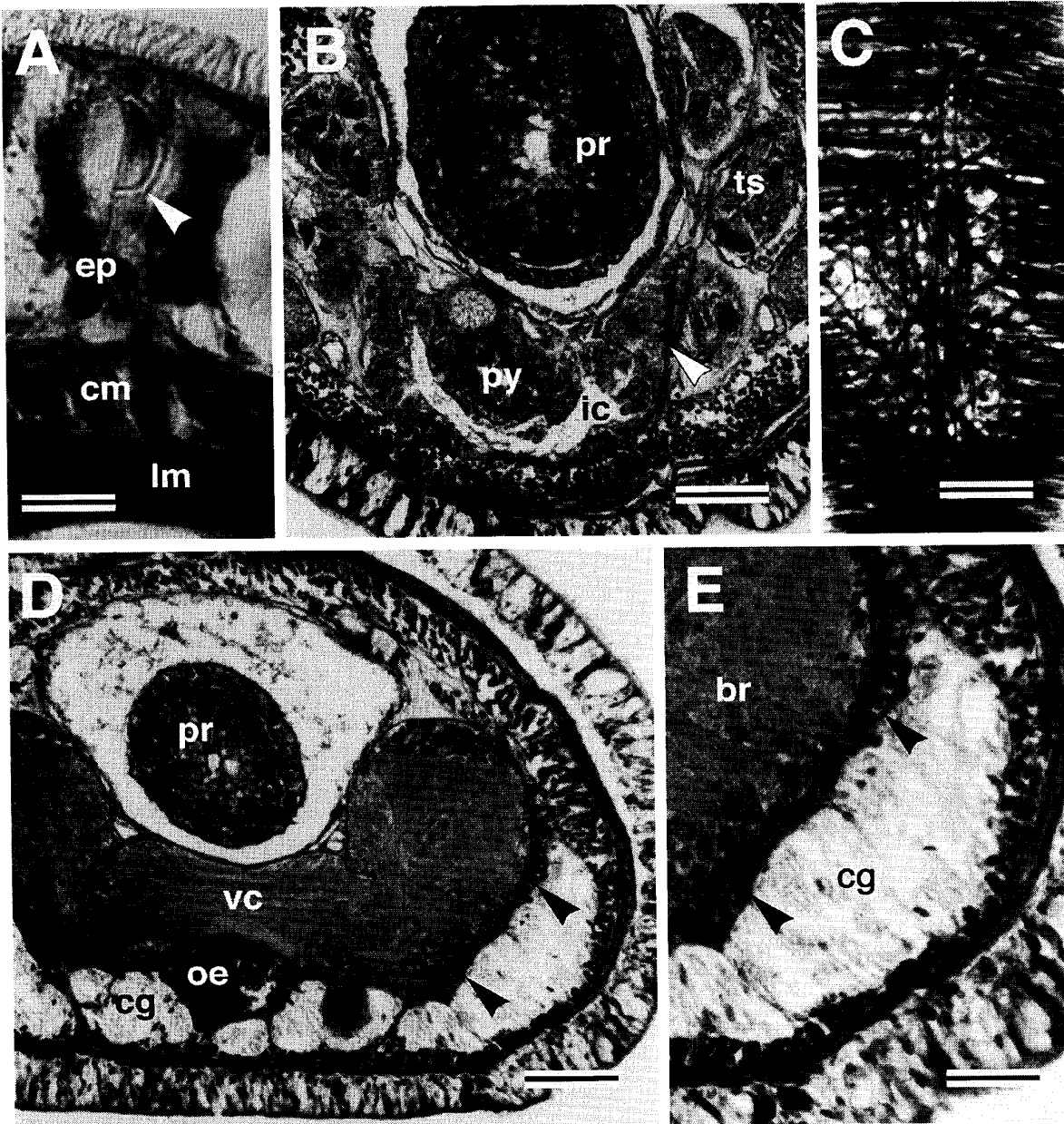
Fig. 7. *Zygonemertes jamsteci* sp. nov. A, longitudinal section to show a sickle-shaped body (indicated by an arrowhead) in the epidermis [ep]; B, transverse section through the intestinal caecum [ic] (an arrowhead indicates a dorso-ventral muscle fibre); C, longitudinal section to show the diagonal muscle fibres; D, E, transverse section through the posterior brain region to show the incompletely divided body-wall longitudinal muscle layer, and an enlarged detail (arrowheads indicate the inner portion of the split longitudinal musculature). Abbreviations: br=brain; cg=cephalic gland; cm=circular muscle layer; lm=longitudinal muscle layer; oe=oesophagus; pr = proboscis; py=pylorus; ts=testis; vc=ventral cerebral commissure. Scale bars: A=10 μm; B, D=50 μm; C, E=25 μm.

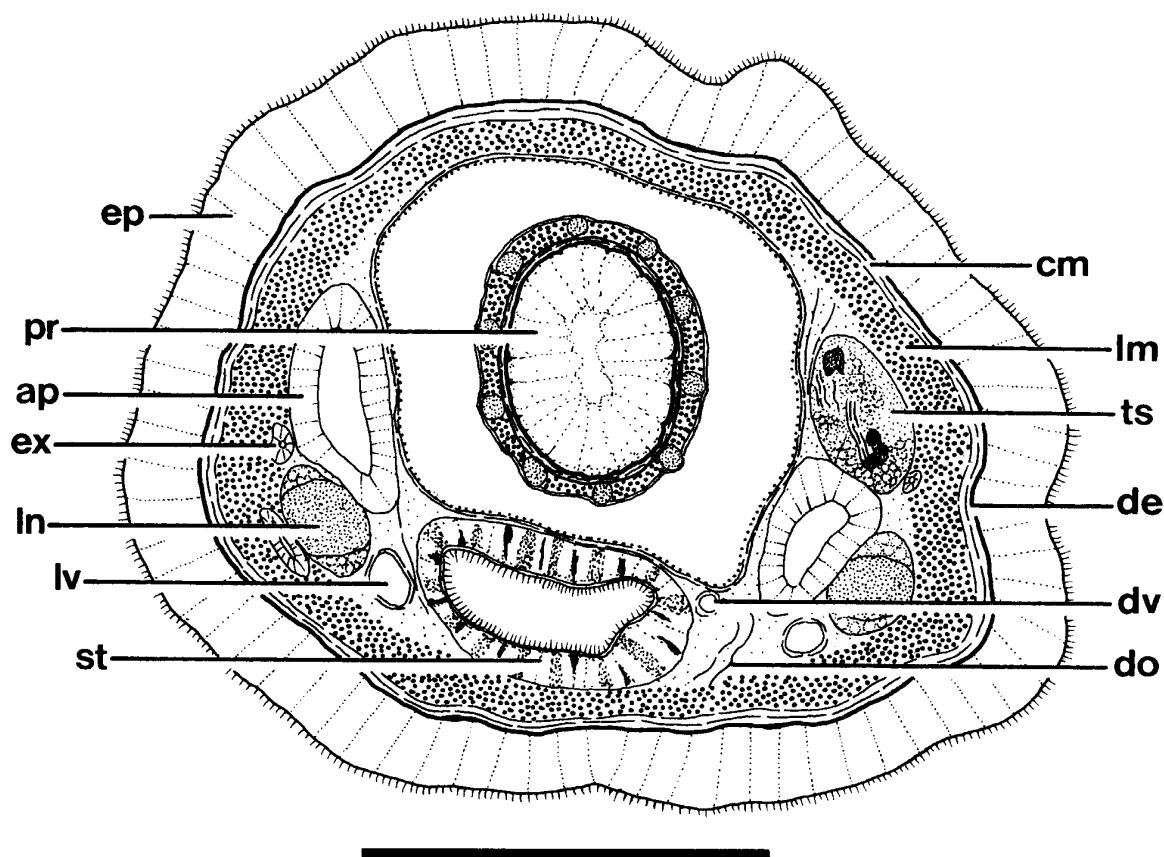134   Hiroshi Kajihara

Fig. 8. *Zygonemertes jamsteci* sp. nov. Transverse section to show the organization of the body in the foregut region. Abbreviations: ap=anterior pouch of the intestinal caecum; cm=circular muscle layer; de=dermis; do=dorsoventral muscle; dv=mid-dorsal blood vessel; ep=epidermis; ex=excretory collecting tubule; lm=longitudinal muscle layer; ln=lateral nerve cord; lv=lateral blood vessel; pr=proboscis; st=stomach; ts=testis. Scale bar=200 μm.

is incompletely divided in the cerebral region. Near the posterior part of the brain the longitudinal layer of the ventral and ventrolateral sides of the body splits off an incomplete inner zone of fibres, separated from the main coat by the posterior extension of the cephalic glands (Fig. 7D, E). This inner layer comprises separate bundles of fibres rather than forming a distinct muscle coat; the muscle bundles lead forward, below and on either side of the brain, to the proboscis insertion. The dorsal portion of the longitudinal musculature is not similarly divided, but gives rise to additional fibres that radiate inward to reinforce the proboscis insertion. The septum is of the mixed type as defined by Gibson (1990). Pre-cerebrally, the remaining longitudinal and circular muscle layers continue to the anterior tip of the head; fibres from the longitudinal layer turn inward to form the cephalic retractor muscles, which pass through the cephalic glands.

Parenchymatous connective tissue is well developed, surrounding the inner organs and being especially conspicuous in the intestinal region.

Proboscis apparatus: The proboscis pore is subterminal. It leads into a short, ciliated chamber, the epithelium of which lacks glandular cells. The rhynchodaeum is lined by a delicate and unciliated epithelium 2–3 μm thick.

The rhynchocoel extends almost to the posterior tip of the body. Its wall contains inner longitudinal and outer circular muscle layers, which are each mostly one or two fibres thick (Fig. 8).

The proboscis is almost as long as, or slightly shorter than, the body (Fig. 6A). The anterior portion is voluminous, attaining about 30% of the body diameter in the retracted state. It is composed of three muscle layers (outer and inner circular, middle longitudinal) (Fig. 9A). Its papillate epithelium is generally 35 μm thick. There are 10 distinct proboscis nerves in all of the sectioned specimens (Fig. 8).

The stylet bulb region possesses two accessory stylet pouches, each containing three to four accessory stylets. The stylet basis ranges from 250 to 370 μm in length, being widest (about 70–75 μm) in its middle portion. The posterior end of the basis is concave (Fig. 9B), but lobed in one specimen (Fig. 6D). The central stylet is smooth and stout, 108–215 μm in length. The stylet/basis ratio varies between 0.43 and 0.58.

Alimentary canal: The oesophagus is short and thin-walled (3–10 μm), and invested with longitudinal muscle fibres. The oesophageal epithelium is neither ciliated nor glandular. The oesophagus is separated from the ventral side of the rhynchodaeum just in front of the proboscis insertion, leading backward beneath the ventral commissure to the stomach.

The stomach (Figs 8, 9C) is histologically divisible into two portions. The short anterior portion, about 72 μm long, possesses an epithelium about 30 μm thick, characterised by densely staining basophilic glands filled with coarsely granular secretions. The main posterior portion has an epithelium, up to 40 μm in maximum thickness, dominated by large, ovoid to pyriform basophilic glands with homogeneous contents. In both portions there are numerous acidophilic and neutrophilic glands. The stomach gradually narrows posteriorly, with reduced height of the epithelium and the number of glandular cells, finally leading to a narrow pylorus (Fig. 7B). The pylorus possesses a ciliated epithelium about 6–10 μm thick, bearing only acidophilic glandular cells. It opens into the dorsal wall of the intestine.

The intestinal caecum (Fig. 7B) is extremely short, ranging from 50 to 102 μm in length. It is anteriorly bifurcated to form a pair of anterior pouches that extend forward to reach the dorsal brain lobes (Fig. 9D). These anterior pouches possess a few shallow, lateral diverticula. The intestinal lateral diverticula are sparingly developed. The anus opens subterminally at the posterior end of the body.

Blood vascular system: The mid-dorsal vessel penetrates the ventral rhynchocoel wall near the rear of the brain to form a vascular plug about 6 μm high and 36 μm wide (Fig. 9C).

The three post-cerebral longitudinal vessels (Fig. 8) are moderately large in diameter but possess thin walls. They extend from the rear of the brain to the posterior end of the body, where they unite to form a supra-intestinal loop close in front of the anus.

Nervous system: The brain is comparatively large, its dorsal and ventral lobes being of similar size. The dorsal and ventral commissures are 18 μm and 38 μm in maximum diameter, respectively. A thin outer neurilemma ensheathes the cerebral ganglia, but there are neither an inner neurilemma nor neurochords. The lateral nerve cords possess a single fibre core; thus, an accessory lateral nerve is absent. The neuropil contains one or two myofibrillae. The lateral nerves send fibres

136   Hiroshi Kajihara

both dorsally and ventrally in the regions of the foregut and intestine (Fig. 9E). The lateral nerves meet posteriorly in a supra-intestinal commissure.

Apical organ and cephalic glands: The single apical organ, consisting of a ciliated pit 25–35 μm in diameter, opens just above the proboscis pore (Fig. 9F). The cephalic glands discharge to the exterior via this apical organ.

The cephalic glands (Figs 7D, E, 9G) occupy much of the space in the pre-cerebral region. Posteriorly, they are bunched into dorsal and ventral lobes. The ventral lobe reaches to the posterior portion of the brain, while the dorsal one is restricted to the anterior margins of the brain. Acidophilic submuscular glands are predominant on the ventral side of the body in the pre-cerebral region.

Sense organs: The eyes are simple, 15–20 μm in diameter. The post-cerebral ocelli are scattered dorsally along the lateral nerve cords.

The cerebral sensory organs (Fig. 7G) are about 45 μm wide and 95 μm high, situated in front of the proboscis insertion. They open ventrolaterally via ciliated cerebral canals, about 30 μm in diameter; the canal epithelium is 10–15 μm thick. The posterior portion of the cerebral organs extends back to reach the underside of the middle portion of the brain.

Excretory system: The excretory system is simple and situated in the stomach region. It comprises thick-walled collecting tubules, 7–9 μm in diameter, mainly located laterally or dorsolaterally to the lateral nerve cords. A single pair of nephridiopores opens ventrolaterally near the rear of the stomach.

Reproductive system: Sexes are separate. The gonads do not regularly alternate with the intestinal lateral diverticula. The anterior-most gonad appears near the posterior part of the stomach.

Testes are arranged above, below, or lateral to the lateral nerve cords. In mature male specimens the testes are 60–100 μm in diameter, containing variably developed sperm (Figs 7B, 9E); the gonoducts pass either above or below the lateral nerve cords (Fig. 9H).

Ovaries are situated mainly above the lateral nerve cords. Every stage of oogenesis can be simultaneously observed in a single specimen. Oocytes appear to originate within the ganglion cell component of the lateral nerve cord (Fig. 10A), as reported by Crandall *et al.* (1998) for *Notogaeanemertes folzae* Riser, 1988. These oocytes can be recognised by their large nuclei containing obvious acidophilic nu-

Fig. 9. *Zygonemertes jamsteci* sp. nov. A, longitudinal section of the anterior portion of the proboscis (arrowheads indicate the inner circular muscle layer of the proboscis); B, photomicrograph of a central stylet and posteriorly concave basis; C, transverse section to show the vascular plug (indicated by an arrowhead); D, transverse section through the posterior brain region to show an anterior pouch (indicated by the arrowhead) of the intestinal caecum reaching above the dorsal ganglion [dg]; E, transver section through the intestinal region to show the lateral nerve cord sending fibres dorso-ventrally (indicated by arrowheads); F, transverse section to show the apical organ [ao]; G, transverse section through the cerebral sensory organ [co]; H, transverse section through the intestinal region (arrowheads indicate the gonoducts, passing above and below the lateral nerve cord [ln]). Abbreviations: cg=cephalic gland; in=intestine; oe=oesophagus; pc=proboscis circular muscle; pl=proboscis longitudinal muscle; pn=proboscis nerve; pp=proboscis papillae; pr=proboscis; rc=rhynchocoel; st=stomach; ts=testis; vg=ventral ganglion. Scale bars: A=10 μm; B=100 μm; C, E=25 μm; D, F, G, H=50 μm.
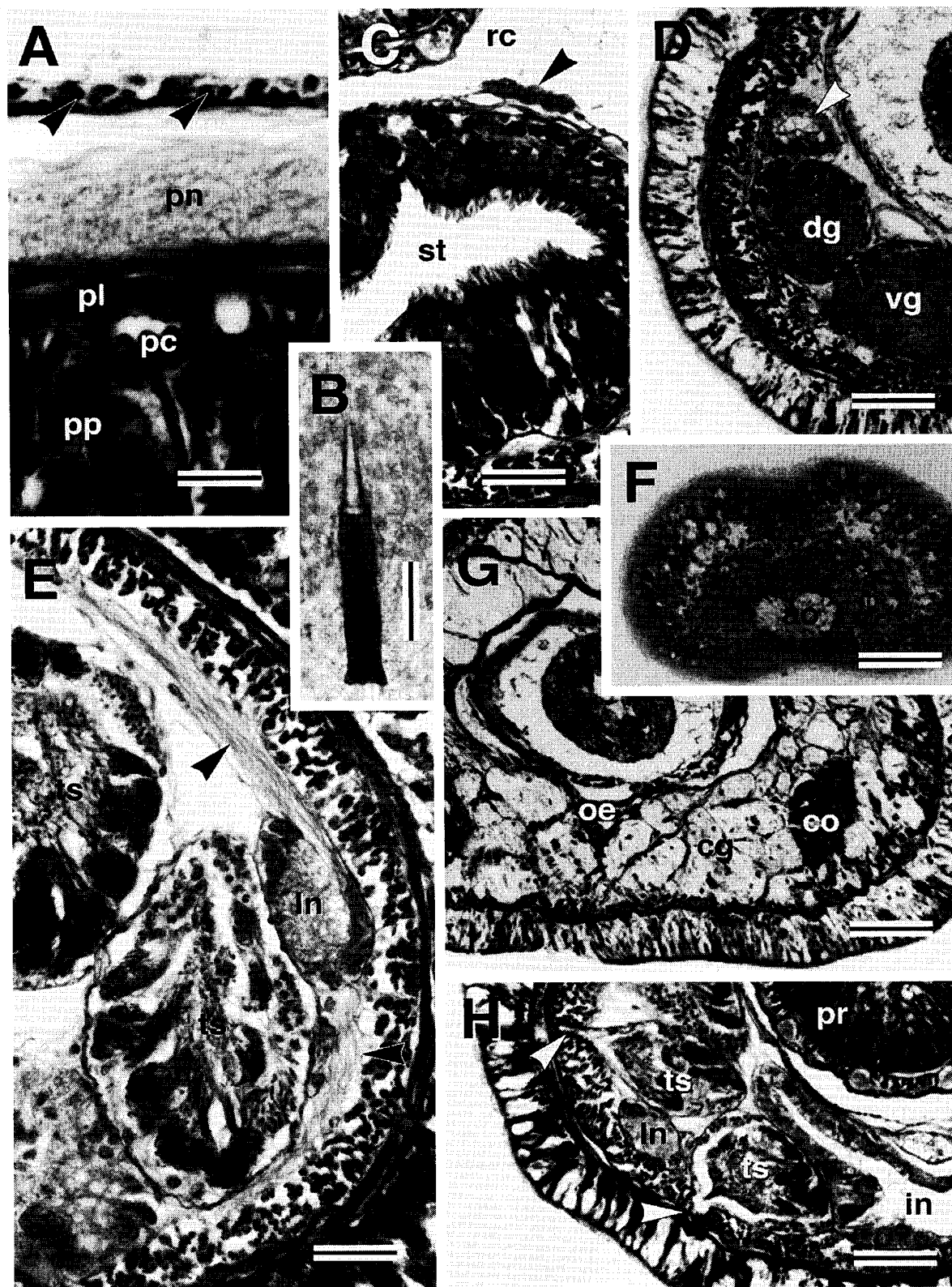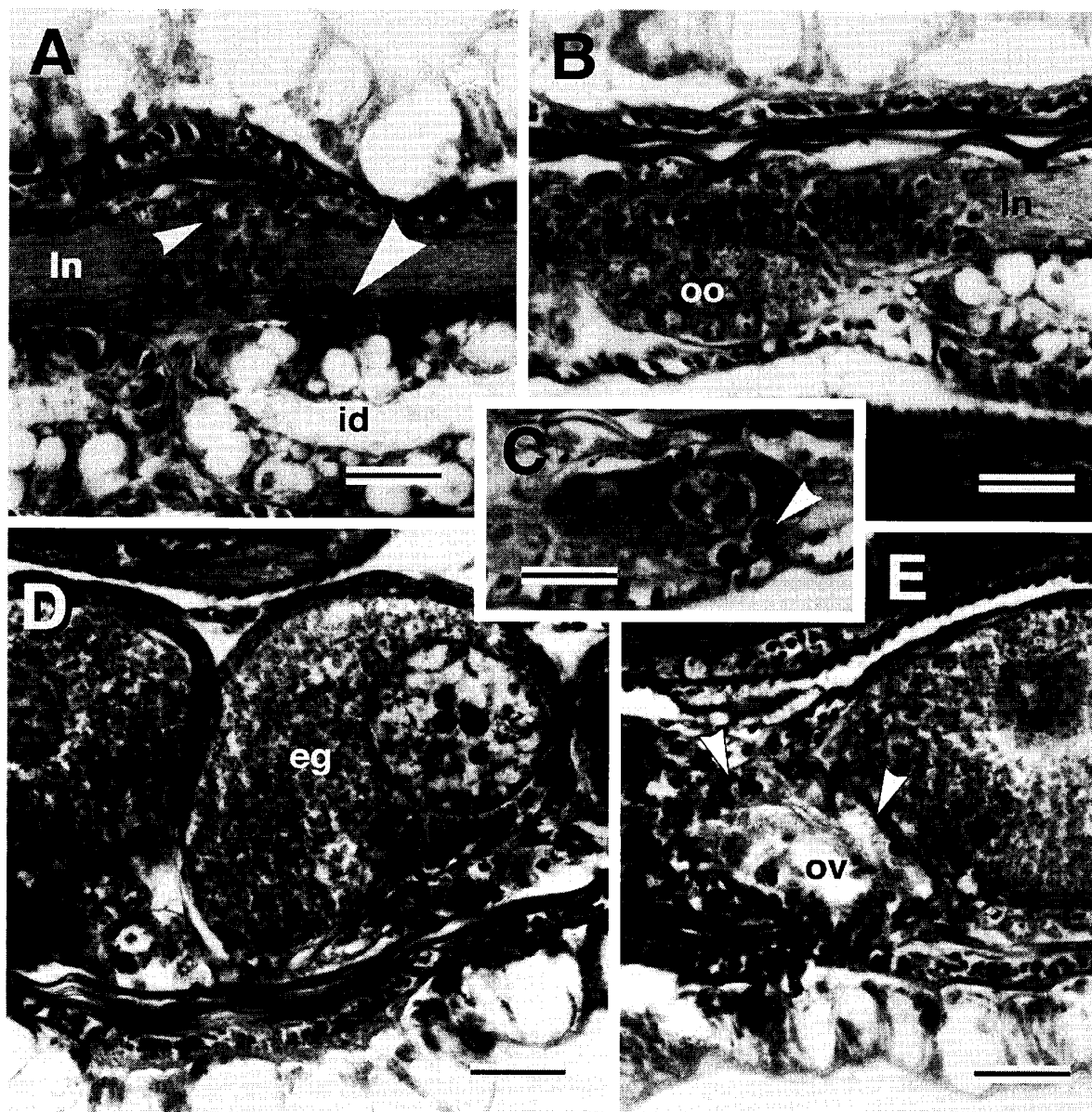
Fig. 10. *Zygonemertes jamsteci* sp. nov. A, longitudinal section to show an oocyte (indicated by the small arrowhead) embedded in the ganglionic cells of the lateral nerve cord [ln] (the larger arrowhead indicates the myofibril); B, longitudinal section to show the proliferated oocytes [oo]; C, longitudinal section to show an oocyte before vitellogenesis (the arrowhead points to the "nuage-like" constriction); D, longitudinal section to show a mature primary oocyte [eg] with yolk granules; E, transverse section to show an empty ovary [ov] (arrowheads indicate its muscular wall). Abbreviations: id=intestinal lateral diverticulum; ln=lateral nerve cord. Scale bars: A–E=25 μm.

cleoli. After an oocyte migrates out of the nerve cord into the adjacent connective tissue, it proliferates up to 20 oocytes per ovary, each with a nuclear size of 10 μm (Fig. 10B); as the oocytes develop, however, the number of oocytes per ovary decreases to one or two. Oocytes in this stage are about 30 μm in diameter with a nucleus of 20 μm in maximum diameter, and they contain reddish-orange-staining
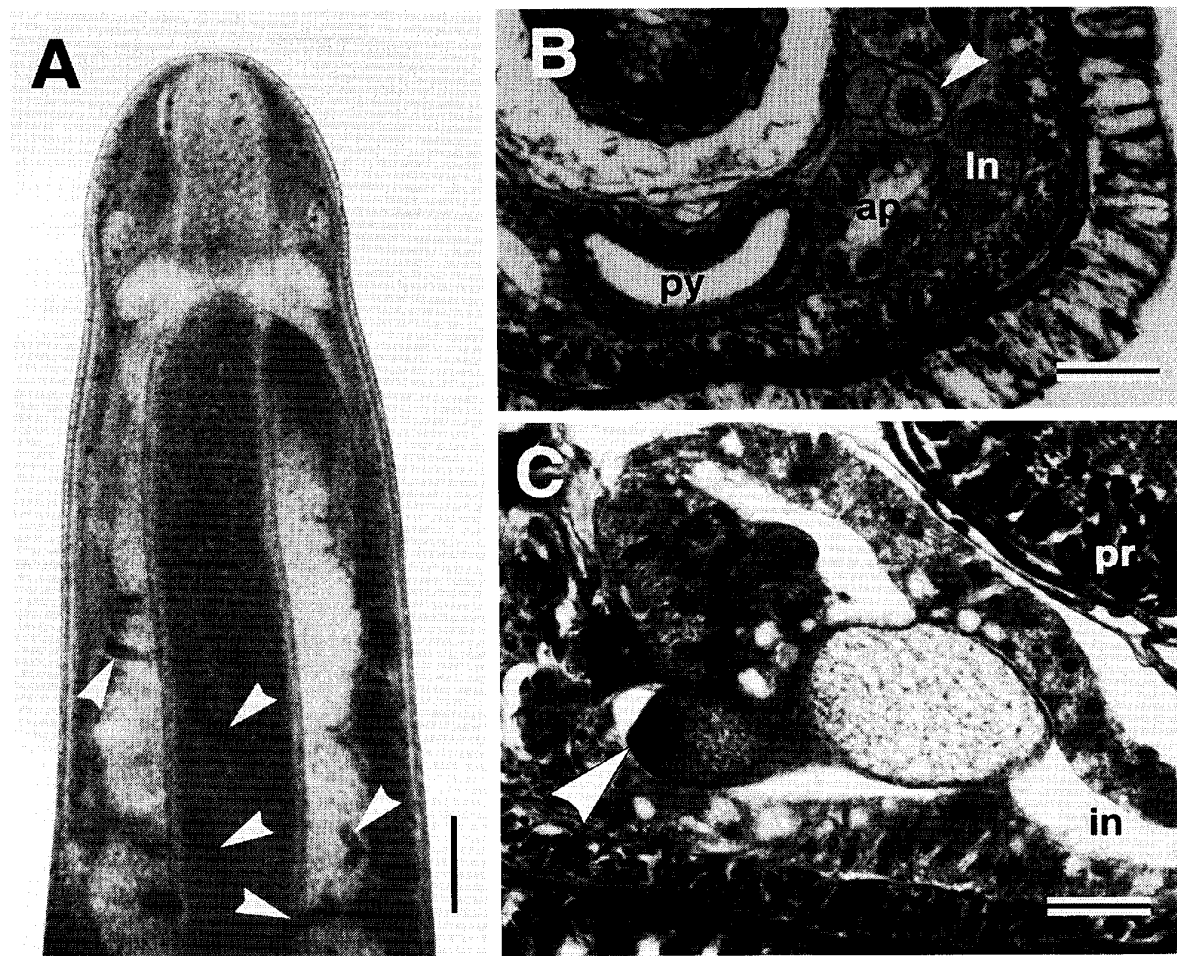
Fig. 11. *Zygonemertes jamsteci* sp. nov. A, photomicrograph of the anterior part of the body of a squeezed specimen, showing unidentified gregarines (indicated by arrowheads); B, transverse section through the pyloric region to show a gregarine (indicated by the arrowhead) in the parenchymatous connective tissue; C, transverse section to show a gregarine in the intestinal lumen (the arrowhead indicates the mucron). Abbreviations: ap=anterior pouch of the intestinal caecum; in=intestine; ln=lateral nerve cord; pr=proboscis; py=pylorus. Scale bars: A=250 μm; B=50 μm; C=25 μm.

granular cytoplasm. This cytoplasm often contains one or two "nuage-like" concretions (Riser 1998), which stain light orange in Mallory (Fig. 10C). A fully mature primary oocyte reaches a diameter of about 90–100 μm, with a germinal vesicle of 40–50 μm (Fig. 10D). Densely acidophilic yolk granules surround the germinal vesicle, its cytoplasm being a thin layer covering the periphery of the oocyte; the egg is thus centrolecithal. The gonoducts pass dorsally to the lateral nerve cords. The walls of empty ovaries are invested with thick muscle fibres (Fig. 10F).

Parasites: Large numbers of unidentified gregarines (Fig. 11A) are distributed both in the parenchymatous connective tissue (Fig. 11B) and in the intestinal lumen (Fig. 11C) in all six sectioned specimens. The gamonts are cylindrical and elongated, reaching up to 240 μm in maximum length. The gamonts have no septum, i.e., they are composed of a single compartment; this suggests that they are

probably acephaline eugregarines. There is a conical mucron on the anterior end of the gamont (Fig. 11C), about 14 μm in diameter, 12 μm high, and staining red in Mallory trichrome. The cytoplasm around the mucron contains basophilic components. The anterior extremity of the gamont, 40–50 μm long, is about 25 μm in diameter. This "neck" region is usually curved (Fig. 11A). Posteriorly, it expands in diameter up to 40 μm; a vesicular nucleus, 20 μm in diameter, is situated a little behind the "neck" region. Further back, the gamont is tapered to end in a rounded tip. Gamonts in syzygy were not observed. Among nemertean species, gregarine parasitism has hitherto been reported from *Cyanophthalma obscura* (Schultze, 1851) (Norenburg 1986), *Potamonemertes percivali* Moore and Gibson, 1973 (Moore and Gibson 1973), and *Baseodiscus delineatus* (Delle Chiaje, 1825) (Kölliker 1848).

Remarks. The anatomy of the present species from Akkeshi Bay, in particular the post-cerebrally distributed ocelli, the intestinal caecum with two anterior pouches, a rhynchocoel extending to the hind end of the body, and lateral nerve cords with no accessory nerves, closely resembles that described herein for *Z. shintai* sp. nov., and the two forms clearly belong to the same genus. The Akkeshi nemerteans also possess sickle-shaped bodies in the epidermis, an apical organ, 10 proboscis nerves, anterior pouches of the intestinal caecum that reach to the brain, a posteriorly concave central stylet basis, and two pairs of cephalic furrows. This combination of features enables the present nemerteans to be distinguished from all the species listed in Table 2 except *Z. virescens* (Verrill, 1879).

Zygonemertes virescens has been reported from both the Atlantic and Pacific coasts of North America (Verrill 1879; Montgomery 1897a, b; Coe 1905a, b, 1940, 1943; Corrêa 1961, 1964; McCaul 1963), Gulf of Mexico (Coe 1951, 1954), and Curaçao (Corrêa 1963). Chernyshev (1991) remarked that *Z. virescens* is also known in Peter the Great Bay, the Sea of Japan, Russia, but did not provide any description of its internal morphology; accordingly, the occurrence of *Z. virescens* in Russian waters is dubious. All of the previous descriptions of *Z. virescens* (e.g., Montgomery 1897a; Coe 1905a, 1943; Corrêa 1961) are inadequate, and a comparison between the present form and these descriptions is difficult. Still, apart from the characters listed in Table 2, the present species from Akkeshi Bay can be distinguished from *Z. virescens* by having a proboscis that is as long as the body. Montgomery (1897a: 2) states that in New England specimens "the thickened proboscis (with the exclusion of its retractor muscle) does not extend quite half the length of the rhynchocoel." Coe (1905a: 215) also mentioned that the "proboscis is little more than half as long [as the body length]" in Californian forms. Further justification for the separation of the present species is the mixed type of pre-cerebral septum. Corrêa (1961: 26) noted that "The proboscidial septum belongs to the closed type" in *Z. virescens* from Florida. It is thus concluded that the present form does not belong to any of the previously known species of *Zygonemertes*, and the new name *Z. jamsteci* is given to it.

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